2 METHODS

This section presents the specific study design and procedures used to implement the Maryland Biological Stream Survey (hereafter referred to as MBSS or the Survey). The study area of concern and the sampling design developed to characterize it are presented, along with field and laboratory methods for each component: water chemistry, physical habitat, fish, benthic macroinvertebrates, amphibians and reptiles, aquatic vegetation, and mussels. Quality assurance and statistical methods are described. This section also summarizes a capture efficiency adjustment for fish and various landscape evaluation methods used to increase the assessment and analysis capabilities of the MBSS. Methods for the formulation of the fish and benthic macroinvertebrate indicators, as well as for the physical habitat indicator, can be found in Chapters 5 and 6 of this report.

2.1 MBSS STUDY DESIGN

The MBSS is a multi-year, probability-based sampling program to:

- assess the status and trends of biological resources in non-tidal streams of Maryland;
- determine how they are affected by acidic deposition and other environmental factors;
- develop an inventory of ecological conditions; and
- aid in targeting restoration activities.

The Survey study area comprises 17 distinct drainage basins across the state (Figure 2-1). Random sampling allows the estimation of unbiased summary statistics (e.g., means, proportions, and their respective variances) for the entire state, a particular basin, or for subpopulations of special interest (e.g., all streams with pH < 5).

Because it would have been cost prohibitive to visit a sufficient number of sites in all basins in a single year, lattice sampling was used to schedule sampling of all basins over a three-year period. Lattice sampling, also known as multi-stratification, is a cost-effective means of allocating effort across time in a large geographic area (Cochran 1977, Jessen 1978). A table, or lattice, was formed by arranging 17 basins in 17 rows, and the years in 3 columns. Lattice sampling was the method used for selecting cells from this 17x3 table so that all basins would be sampled over a three-year period and all basins would have a non-zero probability of being sampled in a given year (Figure 2-1). Although originally included in the design as one of 18 basins originally included in the design, the Conewago basin was not sampled as part of the Survey's random sampling, because its small number of non-tidal stream miles would not permit accurate estimates of basin characteristics. However, in 1997, three sites chosen in a non-random manner in the Conewago basin were sampled using MBSS methods. Similarly, three non-random sites were sampled in the Ocean Coastal basin in 1997 to provide an overview of conditions there. The analyses in this report describe the results of random sampling for the 17 principal basins in Maryland. It does not include the results from supplemental sampling for fish that was conducted to augment the Survey.

The study area was divided into three geographic regions with five to six basins in each: (1) western, (2) central, and (3) eastern. This geographic stratification facilitated the effective use of three sampling crews from the different regions. Two basins were randomly selected (without replacement) from each region for sampling each year. One randomly selected basin in each region was visited twice, in order to quantify between-year variability in the response variables. A new set of randomly-selected sites was chosen for the repeat year. This controlled selection of cells from the lattice allowed estimation of average condition for all cells; (i.e., the average condition for all basins over a three-year period).

The sampling frame for the Survey was constructed by overlaying basin boundaries on a map of all blue-line stream reaches in the study area as digitized on a U.S. Geological Survey 1:250,000 scale topographic map. This sample frame was similar to that used by the earlier Maryland Synoptic Stream Chemistry Survey (MSSCS) conducted in 1987 (Knapp and Saunders 1987, Knapp et al. 1988). The Strahler convention (Strahler 1957) was used for ranking stream reaches by order; first-order reaches, for example, are the most upstream reaches in the branching stream system. Sampling was restricted to non-tidal, third-order and smaller stream reaches, excluding impoundments that were non-wadable or that substantially altered the riverine nature of the reach (Kazyak 1994). Together, these first- through third-order streams comprise about 90% of all stream and river miles in Maryland. Stream reaches were further divided into non-overlapping, 75-meter segments; these segments were the elementary sampling units from which biological, water chemistry, and physical habitat data were collected.

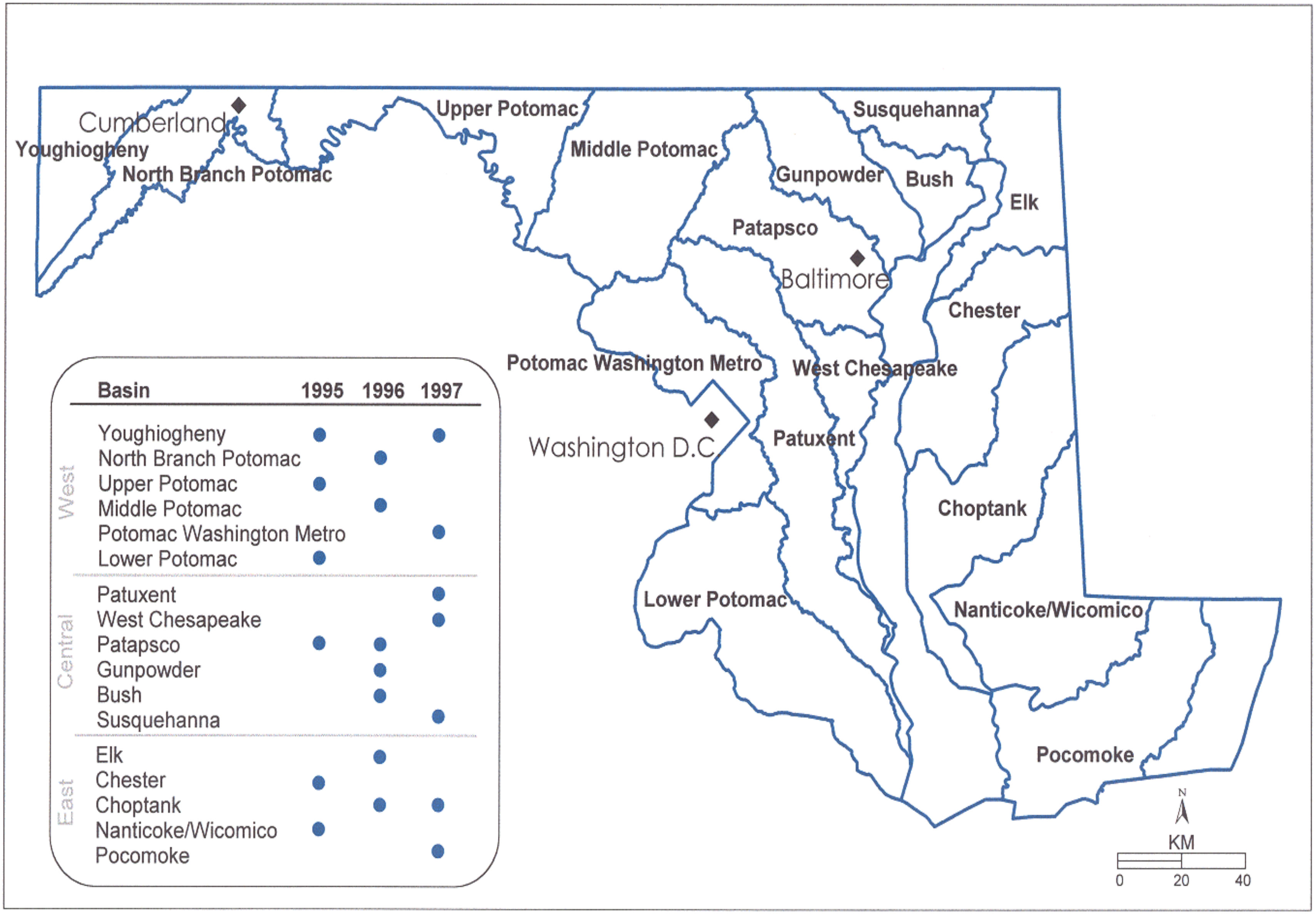

Figure 2-1. Basins in the MBSS study area and the years scheduled for sampling in the 1995-1997 survey

The 1995-97 MBSS study design was based on stratified random sampling of segments within each basin; each basin was stratified by stream order (Figure 2-2). Within a stream order, the number of segments sampled per basin is proportional to the number of stream miles in the basin (Appendix B, Table B-1). To achieve the target number of samples per stream order within each basin, a given number of segments were randomly selected from each basin and ranked in order of selection. In all basins, extra segments were selected as a contingency against loss of sampling sites from restricted access to selected streams or from streams that were dry, too deep, or otherwise unsampleable owing to field conditions. In some basins, where only a small number of sites would have been selected using this method, additional random sites were selected to increase sample size. These extra sites (selected at random using the method described above) were used to provide better basinwide estimates; they were not included in the estimates of statewide conditions.

Permissions were obtained to access privately owned land adjacent to or near each stream segment. The procedures for obtaining permissions are described in Chaillou (1995). Because landowner permissions were obtained in a synoptic fashion and some variation in these rates occurred, we obtained more permissions than were needed for the Survey. Only the highest ranking sites were sampled until the target goal for that basin was reached. For the three year study, the success rate for obtaining permission to access stream sampling segments was high. Eighty-eight percent of sites that were targeted for permission were sampled (Table 2-1). Reasons for permission denial varied widely and generally reflected the preferences of individual landowners regarding property access, rather than any specific types of land. In rare cases, permission denial may affect the interpretation of MBSS estimates, but only where denials occur in streams with characteristics that differ from the general population of streams. In one example of potential bias, several sites with known coal mining activities in the North Branch Potomac basin denied permission to sample, likely under representing the proportion of acid mine drainage in the population.

Table 2-1. Landowner permission success rates for basins sampled in the 1995-1997 MBSS

Basin	Number of Stream Segments Targeted as Potential Sample Sites	Success Rate
Youghiogheny 1995	71	75%
Youghiogheny 1997	46	78%
North Branch Potomac	90	86%
Upper Potomac	99	87%
Middle Potomac	165	87%
Potomac Washington Metro	94	97%
Lower Potomac	91	77%
Patuxent	103	93%
West Chesapeake	53	91%
Patapsco 1995	96	86%
Patapsco 1996	89	87%
Gunpowder	66	89%
Bush	45	87%
Susquehanna	45	94%
Elk	41	78%
Chester	82	93%
Choptank 1996	44	93%
Choptank 1997	33	94%
Nanticoke/Wicomico	62	100%
Pocomoke	58	94%
TOTAL	1,473	88%

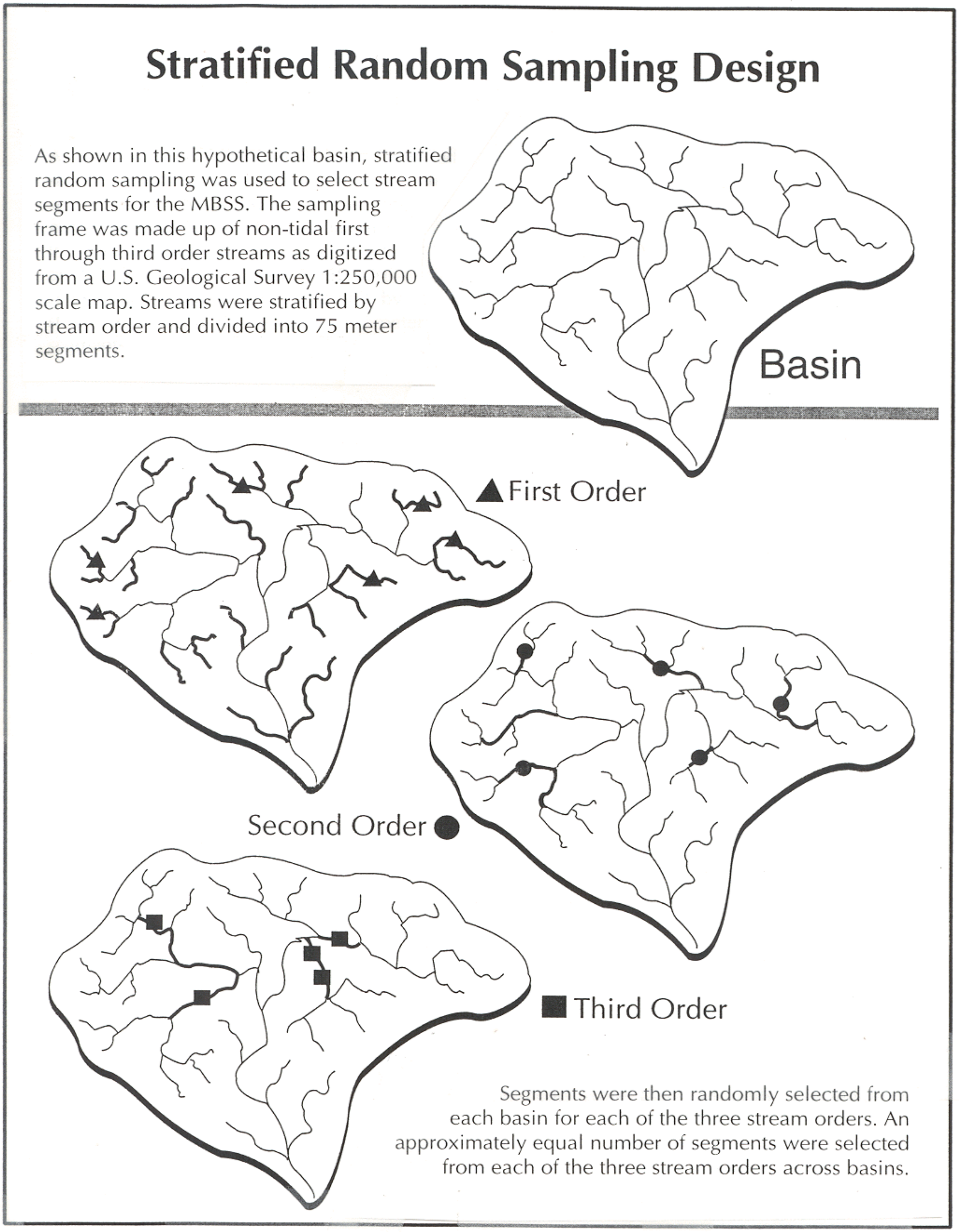

Figure 2-2. MBSS stratified random sampling design

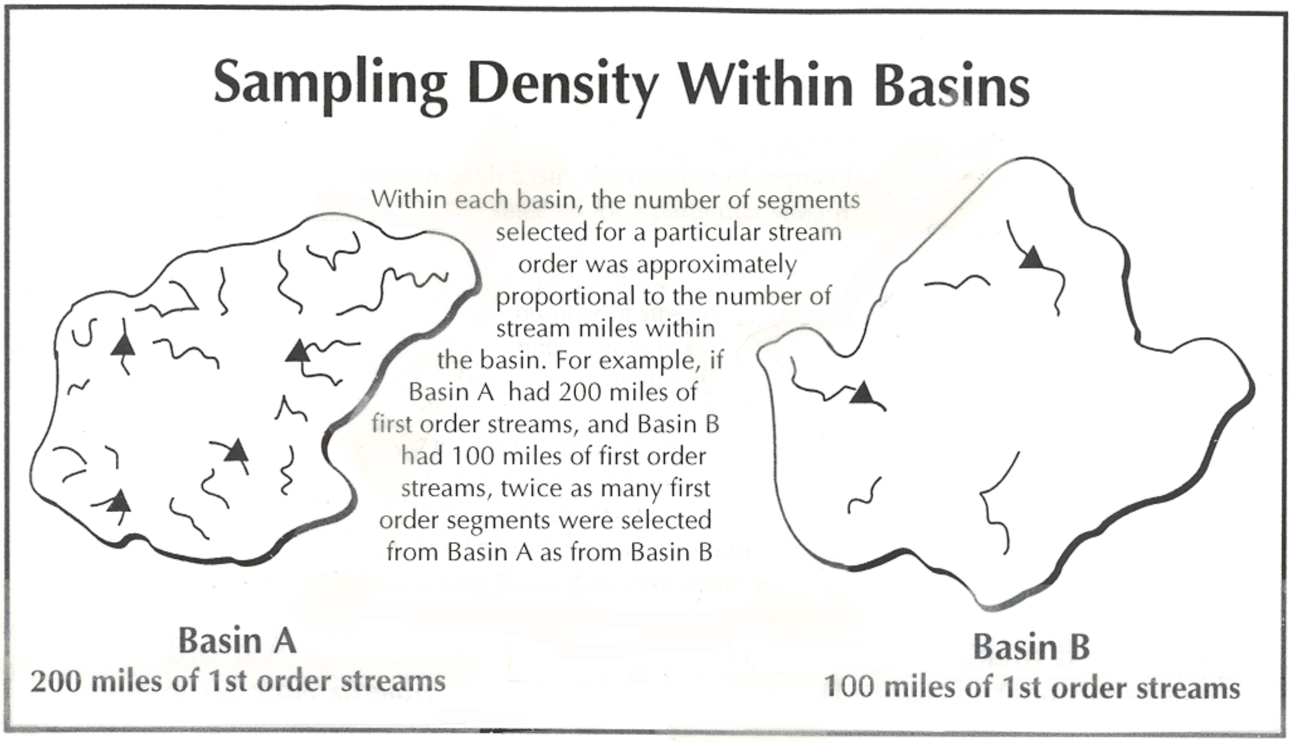

Figure 2-2. Continued

2.2 DESCRIPTION OF STUDY AREA

The Survey encompasses first-, second-, and third-order streams in Maryland, as determined from the 1:250,000 scale base map. It is important that the stream systems included in the Survey were precisely described in terms of the extent, location, and order of each type of stream. For the 17 basins sampled in the Survey, the number of first- through third-order stream miles ranged from 186 (Bush) to 1,102 (Middle Potomac) (Appendix B, Table B-1). The number of first-order stream miles (5,820) was about four times the number of second-order and eight times the number of third-order stream miles. Only by reference to these "total stream miles" can estimates of the percentage of the resource with specific attributes be converted to the total amount of the resource.

2.3 FIELD AND LABORATORY METHODS

In all, 955 stream segments were successfully sampled in the spring during 1995-1997; of those, 905 were also sampled in summer (Figure 2-3; Appendix B, Table B-2). Benthic macroinvertebrate and water quality sampling were conducted in spring, when the benthos are thought to be reliable indicators of environmental stress (Plafkin et al. 1989) and when acid deposition effects are often the most pronounced. Fish, amphibians and reptiles, aquatic vegetation, and mussel sampling, along with physical habitat evaluations, were conducted at 905 segments during the low-flow period in summer. Fish community composition tends to be stable during summer, and low flow is advantageous for electrofishing. Because low-flow conditions in summer may be a primary factor limiting the abundance and distribution of fish populations, habitat assessments were performed during the summer. The sample size in summer is lower than in spring because some streams were dry in summer or were, in rare cases, otherwise unsampleable.

To reduce temporal variability, sampling during spring and summer was conducted within specific, relatively narrow time intervals, referred to as index periods (Janicki et al. 1993). These index periods were defined by degree-day limits for specific parts of the state. This approach provided a synoptic assessment of the current status of stream biota, water quality, and physical habitat in the 17 basins sampled. The spring index period was the time period between approximately March 1 and May 1, with end of the index period determined by degree-day accumulation as

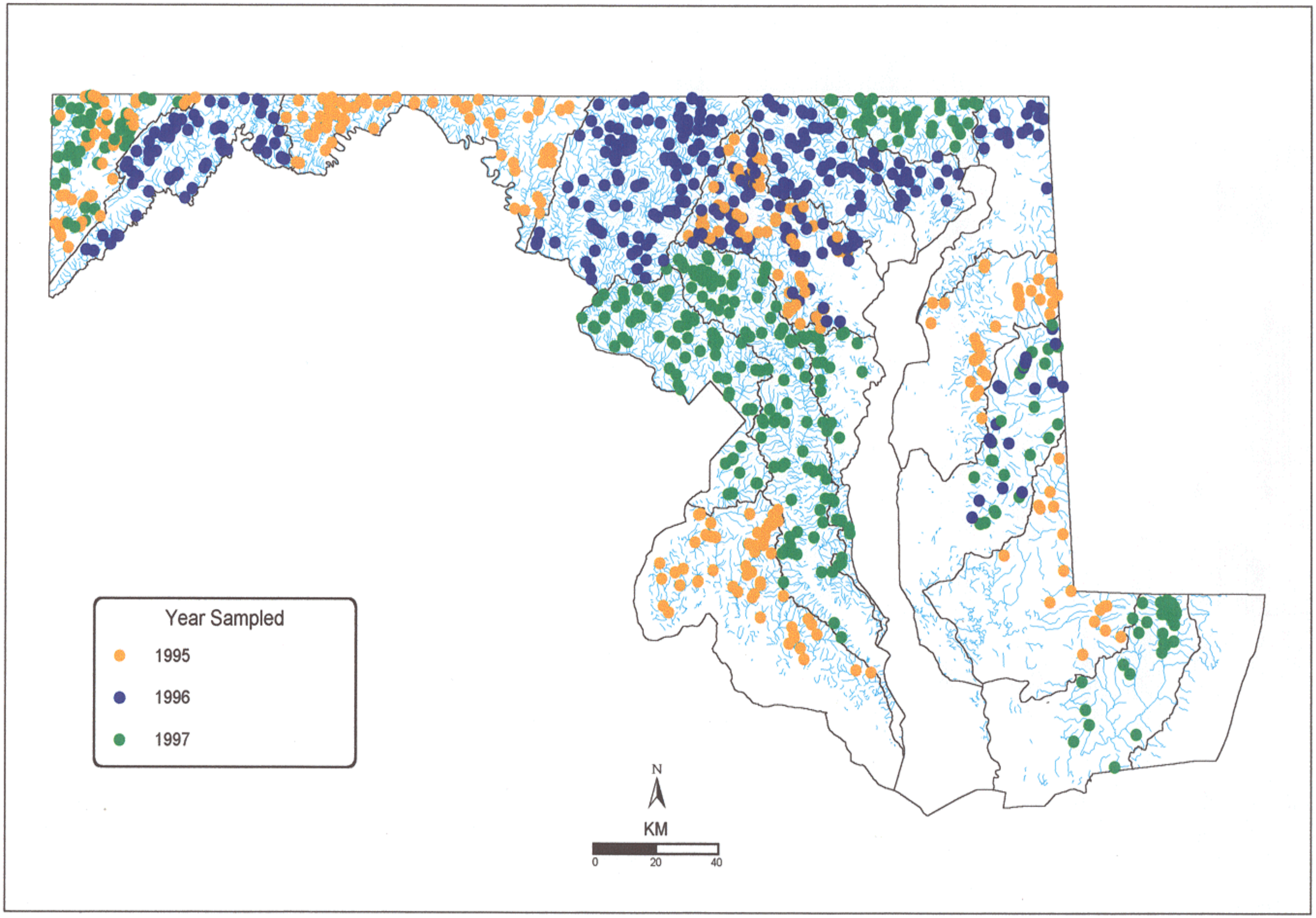

Figure 2-3. Randomly selected sites sampled in the 1995-1997 MBSS (955 sites)

specified in Hilsenhoff (1987). In reality, most spring samples (78%) were collected in March, well before degree-day accumulation limits were approached. The summer index period was between June 1 and September 30 (Kazyak 1994).

2.3.1 Data Collection and Measurement

Field sampling followed procedures specified in the MBSS sampling manual (e.g., Kazyak 1996). A summary of the variables measured and the field and laboratory methods used to conduct the sampling follows.

2.3.1.1 Water Chemistry

During the spring index period, water samples were collected at each site for analysis of pH, acid neutralizing capacity (ANC), conductivity, sulfate, nitrate-nitrogen, and dissolved organic carbon (DOC). These variables describe basic water quality conditions with an emphasis on factors related to acidic deposition.

Grab samples were collected in one-liter bottles for analysis of all analytes except pH. Water samples for pH were collected with 60 ml syringes, which allowed purging of air bubbles to minimize changes in carbon dioxide content (EPA 1987). Samples were stored on wet ice and shipped on wet ice to the analytical laboratory within 48 hours. Laboratory analyses were carried out by the University of Maryland's Appalachian Laboratory in Frostburg.

Chemical analysis of water samples followed standard methods described in EPA's Handbook of Methods for Acid Deposition Studies (EPA 1987). These methods are summarized in Table 2-2. EPA protocols were followed, except that ANC sample volume was reduced to 40 ml to ease sample handling. Routine daily quality control (QC) checks included processing duplicate, blank, and calibration samples according to EPA guidelines for each analyte. Field duplicates were taken at 5% of all sites. Routine QC checks helped to identify and correct errors in sampling routines or instrumentation at the earliest possible stage.

During the summer index period, *in situ* measurements of dissolved oxygen (DO), pH, temperature, and conductivity were collected at each site to further characterize existing water quality conditions that might influence biological communities. Measurements were made at an undisturbed section of the segment, usually in the middle of the stream channel, using electrode probes. Instruments were calibrated daily and calibration logbooks were maintained to document instrument performance.

2.3.1.2 Benthic Macroinvertebrates

Benthic macroinvertebrates were collected to provide a qualitative description of the community composition at each sampling site (Kazyak 1996). Sampling was conducted during the spring index period. Benthic community data were collected for the purpose of calculating biological metrics, such as those described in EPA's Rapid Bioassessment Protocols (Plafkin et al. 1989), and use as an indicator of biological integrity for Maryland streams.

At each segment, a 600 micron mesh "D" net was used to collect organisms from habitats likely to support the greatest taxonomic diversity. A riffle area was preferred, but other habitats were also sampled using a variety of techniques including kicking, jabbing, and gently rubbing hard surfaces by hand to dislodge organisms. If available, other habitat types were sampled, including rootwads, woody debris, leaf packs, macrophytes, and undercut banks. Each jab covered one square foot, and a total of approximately 2.0 m^2 (20 square feet) of combined substrates was sampled and preserved in 70% ethanol. In the laboratory, the preserved sample was transferred to a gridded pan and organisms were picked from randomly selected grid cells until the cell that contained the 100th individual (if possible) was completely picked. Some samples had fewer than 100 individuals. The benthic macroinvertebrates were identified to genus, or lowest practicable taxon, in the laboratory.

2.3.1.3 Fish

Fish were sampled during the summer index period using double-pass electrofishing within 75-meter stream segments. Block nets were placed at each end of the segment and direct current backpack electrofishing units were used to sample the entire segment. An attempt was made to thoroughly fish each segment, sampling the entire stream segment. A consistent effort was applied over the two passes. This sampling approach allowed calculation of several metrics useful in calculating a biological index and produced estimates of fish species abundance.

Table 2-2. Analytical methods used for water chemistry samples collected during the spring index period. See EPA (1987) for details

Analyte (units)	Method	Instrument	Detection Limit	Holding Time (days)
pH (standard units)	EPA Sec. 19.0	Closed system using Orion 611 pH meter equipped with Orion 08104 Ross combination electrode and Hellman chamber	0.01	7
Specific Conductance (μmho/cm)	EPA 120.1	YSI 32 equipped with 3403 conductivity cell (1.0 cm/sec cell constant)	NA	14
Acid Neutralizing Capacity (μeq/l)	EPA Sec. 5.0 modified	Titration (modified Gran analysis) using Orion 611 pH meter	NA	14
Dissolved Organic Carbon (mg/l)	EPA 415.1	Doorman DC-80 carbon analyzer	1.0	14
Sulfate (mg/l)	EPA 300.0	Danaus 2001i ion chromatography (with upgrade)	0.206	14
Nitrate Nitrogen (mg/l)	EPA 300.0	Danaus 2001i ion chromatography (with upgrade)	0.013	14

NA = Not Applicable

In small streams, a single electrofishing unit was used. In larger streams, two to five units were employed to effectively sample the site. Captured fish were identified to species, counted, weighed, and released. Any individuals that could not be identified to species were retained for laboratory confirmation. For each pass, all individuals of each gamefish species (defined as trout, bass, walleye, pike, chain pickerel, and striped bass) were measured for total length and examined for visible external pathologies or anomalies. For nongame species, up to 100 fish of each species (from both passes) were examined for visible external pathologies or anomalies. For each pass, all non-game species were weighed together for an aggregate biomass measurement; gamefish were also weighed in aggregate to the nearest 10 g.

Electrofishing was also conducted at supplemental, non-randomly selected sites during the summer. The presence of each species of fish was recorded for these segments to provide additional qualitative information on fish distributions. Sampling effort at most supplemental sites was based on doubling the elapsed time since the last species was recorded or a minimum of 600 seconds of electrofishing effort.

After processing the fish collection in the field, voucher specimens were retained for each species not previously collected in the drainage basin. In addition, all individuals which could not be positively identified in the field were retained. The remaining fish were released. All voucher specimens and fish retained for positive identification in the laboratory were examined and verified by the MBSS Quality Assurance Officer or ichthyologists at Frostburg State University, Frostburg, Maryland or the Smithsonian Institution, Washington, DC.

2.3.1.4 Amphibians and Reptiles

At each sample segment, amphibians and reptiles were identified and the presence of observed species was recorded during the summer index period. A search of the riparian area was conducted within 5 meters of the stream on both sides of the 75-meter segment. Any amphibians and reptiles collected during the electrofishing of the stream segment were also included in the species list. Individuals were identified to species when possible. Voucher specimens and individuals not positively identifiable in the field were retained for examination in the laboratory and confirmation by herpetologists at the Smithsonian Institution, Washington, DC, and/or Towson University, Towson, Maryland.

2.3.1.5 Mussels

During the summer index period, freshwater mussels were sampled qualitatively by examining each 75-meter stream segment for their presence. Mussels were identified to species, their presence recorded, and individuals released. Species not positively identifiable in the field were retained for confirmation by U.S. Geological Survey (USGS) Biological Resources Division staff.

2.3.1.6 Aquatic Vegetation

During the summer index period, aquatic vegetation was sampled qualitatively by examining each 75-meter stream segment for the presence of aquatic plants. Plants were identified to species and their (if possible) presence recorded for each site. While the primary objective was to document the presence of submerged aquatic vegetation (SAV), emergent vegetation was also recorded when encountered. Species not positively identifiable in the field were retained for examination in the laboratory and confirmation by DNR's staff expert on SAV. Due to the difficulty in long-term preservation, no permanent vouchers of aquatic vegetation were retained.

2.3.1.7 Physical Habitat

Habitat assessments were conducted at all stream segments as a means of assessing the importance of physical habitat to the biological integrity and fishability of freshwater streams in Maryland. Procedures for habitat assessments (Kazyak 1996) were derived from two currently used methodologies: EPA's Rapid Bioassessment Protocols (RBPs) (Plafkin et al. 1989), as modified by Barbour and Stribling (1991), and the Ohio EPA's Qualitative Habitat Evaluation Index (QHEI) (Ohio EPA 1987, Rankin 1989). Guidelines for qualitative habitat assessment scoring are listed in Table 2-3. A number of characteristics (instream habitat, epifaunal substrate, velocity/depth diversity, pool/glide/eddy quality, riffle/run quality, channel alteration, bank stability, embeddedness, channel flow status, and shading) were assessed qualitatively, based on visual observations within each 75-meter sample segment. Riparian zone vegetation width was estimated to the nearest meter, up to 50 meters from the stream. Additional observations of the surrounding area were used to assign ratings for aesthetic value (based on visible signs of human refuse at a site) and remoteness (based on distance from the nearest road, accessibility, and evidence of human activity). Also recorded were the presence or absence of various stream features including substrate types, various morphological characteristics, beaver ponds, point sources, and stream channelization. Local land uses visible from the stream segment and riparian vegetation type were also noted.

Several additional physical characteristics were measured quantitatively to further characterize the habitat for each segment (see Kazyak 1996 for details). Quantitative measurements of the segment included maximum depth, stream gradient, velocity, thalweg depth, number of functional rootwads, number of functional large woody debris, wetted width, sinuosity, and overbank flood height. A velocity/depth profile was measured or other data were collected to enable calculation of discharge.

Recognizing that water temperature is an important factor affecting stream condition (but one that varies daily and seasonally), the Survey deployed temperature loggers at 220 sites in five basins during the sample year 1997. The basins sampled were: the Choptank, Susquehanna, Potomac Washington Metro, Patuxent, and Pocomoke basins. Onset Computer Corporation Optic Stowaway model temperature loggers were anchored in each sample site during the summer index period. They recorded the water temperature every 15 minutes from June 15 until mid-September.

2.3.2 Data Management

All crews used standardized pre-printed data forms developed for the Survey to ensure that all data for each sampling segment were recorded and standard units of measure were used (Kazyak 1996). Using standard data forms facilitated data entry and minimized transcription error. The field crew leader and a second reviewer checked all data sheets for completeness and legibility before leaving each sampling location. Original data sheets were sent to the Data Management Officer for further review and data entry, while copies were retained by the field crews.

A custom database application, in which the input module was designed to match each of the field data sheets, was used for data entry. Data were independently entered into two databases and compared using a computer program as a quality-control procedure. Differences between the two databases were resolved from original data sheets or through discussions with field crew leaders.

Table 2-3. Guidelines for qualitative habitat assessment (Kazyak 1996)

MBSS Habitat Assessment Guidance Sheet				
Habitat Parameter	**Optimal 16-20**	**Sub-Optimal 11-15**	**Marginal 6-10**	**Poor 0-5**
1. Instream Habitat[a]	Greater than 50% mix of a variety of cobble, boulder, submerged logs, undercut banks, snags, rootwads, aquatic plants, or other stable habitat	30-50% mix of stable habitat. Adequate habitat	10-30% mix of stable habitat. Habitat availability less than desirable	Less than 10% stable habitat. Lack of habitat is obvious
2. Epifaunal Substrate[b]	Preferred substrate abundant, stable, and at full colonization potential (riffles well developed and dominated by cobble; and/or woody debris prevalent, not new, and not transient)	Abund. of cobble with gravel &/or boulders common; or woody debris, aquatic veg., undercut banks, or other productive surfaces common but not prevalent /suited for full colonization	Large boulders and/or bedrock prevalent; cobble, woody debris, or other preferred surfaces uncommon	Stable substrate lacking; or particles are over 75% surrounded by fine sediment or flocculent material
3. Velocity/Depth Diversity[c]	Slow (<0.3 m/s), deep (>0.5 m); slow, shallow (<0.5 m); fast (>0.3 m/s), deep; fast, shallow habitats all present	Only 3 of the 4 habitat categories present	Only 2 of the 4 habitat categories present	Dominated by 1 velocity/depth category (usually pools)
4. Pool/Glide/Eddy Quality[d]	>50% pool/glide/eddy habitat; both deep (>.5 m)/shallows (<.2 m) present; complex cover/&/or depth >1.5 m	10-50% pool/glide/eddy habitat, with deep (>0.5 m) areas present; or >50% slow water with little cover	<10% pool/glide/eddy habitat, with shallows (<0.2 m) prevalent; slow water areas with little cover	Pool/glide/eddy habitat minimal, with max depth <0.2 m, or absent completely
5. Riffle/Run Quality[e]	Riffle/run depth generally >10 cm, with maximum depth greater than 50 cm (maximum score); substrate stable (e.g. cobble, boulder) & variety of current velocities	Riffle/run depth generally 5-10 cm, variety of current velocities	Riffle/run depth generally 1-5 cm; primarily a single current velocity	Riffle/run depth < 1 cm; or riffle/run substrates concreted
6. Channel Alteration[f]	Little or no enlargement of islands or point bars; no evidence of channel straightening or dredging; 0-10% of stream banks artificially armored or lined	Bar formation, mostly from coarse gravel; and/or 10-40% of stream banks artificially armored or obviously channelized	Recent but moderate deposition of gravel and coarse sand on bars; and/or embankments on both banks; and/or 40-80% of banks artificially armored; or channel lined in concrete	Heavy deposits of fine material, extensive bar development; OR recent channelization or dredging evident; or over 80% of banks artificially armored
7. Bank Stability[g]	Upper bank stable, 0-10% of banks with erosional scars and little potential for future problems	Moderately stable. 10-30% of banks with erosional scars, mostly healed over. Slight potential in extreme floods	Moderately unstable. 30-60% of banks with erosional scars and high erosion potential during extreme high flow	Unstable. Many eroded areas. "Raw" areas frequent along straight sections and bends. Side slopes >60° common
8. Embeddedness[h]	Percentage that gravel, cobble, and boulder particles are surrounded by line sediment or flocculent material.			
9. Channel Flow Status[i]	Percentage that water fills available channel			
10. Shading[j]	Percentage of segment that is shaded (duration is considered in scoring). 0% = fully exposed to sunlight all day in summer; 100% = fully and densely shaded all day in summer			
11. Riparian Buffer[k]	Minimum width of vegetated buffer in meters; 50 meters maximum; see back of Habitat Assessment Data Sheet for buffer type and land cover immediately adjacent to buffer			

Table 2-3. Continued

Habitat Parameter	Optimal (16-20)	Sub-Optimal (11-15)	Marginal (6-10)	Poor (0-5)
12. Aesthetic Rating[l]	Little or no evidence of human refuse present; vegetation visible from stream essentially in a natural state	Human refuse present in minor amounts; and/or channelization present but not readily apparent; and/or minor disturbance of riparian vegetation	Refuse present in moderate amounts; and/or channelization readily apparent; and/or moderate disturbance of riparian vegetation	Human refuse abundant and un-sightly: and/or extensive unnatural channelization; and/or nearly complete lack of vegetation
13. Remoteness[m]	Stream segment more than 1/4 mile from nearest road; access difficult and little or no evidence of human activity	Stream segment within 1/4 of but not immediately accessible to roadside access by trail; site with moderately wild character	Stream within 1/4 mile of roadside and accessible by trail; anthropogenic activities readily evident	Segment immediately adjacent to roadside access; visual , olfactory, and/or auditory displeasure experienced

a) **Instream Habitat** Rated based on perceived value of habitat to the fish community. Within each category, higher scores should be assigned to sites with a variety of habitat types and particle sizes. In addition, higher scores should be assigned to sites with a high degree of hypsographic complexity (uneven bottom). In streams where ferric hydroxide is present, instream habitat scores are not lowered unless the precipitate has changed the gross physical nature of the substrate. In streams where substrate types are favorable but flows are so low that fish are essentially precluded from using the habitat, low scores are assigned. If none of the habitat within a segment is useable by fish, a score of zero is assigned.

b) **Epifaunal Substrate** Rated based on the amount and variety of hard, stable substrates usable by benthic macroinvertebrates. Because they inhibit colonization, flocculent materials or fine sediments surrounding otherwise good substrates are assigned low scores. Scores are also reduced when substrates are less stable.

c) **Velocity/Depth Diversity** Rated based on the variety of velocity/depth regimes present at a site (slow-shallow, slow-deep, fast-shallow, and fast-deep). As with embeddedness, this metric may result in lower scores in low-gradient streams but will provide a statewide information on the physical habitat found in Maryland streams.

d) **Pool/Glide/Eddy Quality** Rated based on the variety and spatial complexity of slow- or still-water habitat within the sample segment. It should be noted that even in high-gradient segments, functionally important slow-water habitat may exist in the form of larger eddies. Within a category, higher scores are assigned to segments which have undercut banks, woody debris or other types of cover for fish.

e) **Riffle/Run Quality** Rated based on the depth, complexity, and functional importance of riffle/run habitat in the segment, with highest scores assigned to segments dominated by deeper riffle/run areas, stable substrates, and a variety of current velocities.

f) **Channel Alteration** Is a measure of large-scale changes in the shape of the stream channel. Channel alteration includes: concrete channels, artificial embankments, obvious straightening of the natural channel, rip-rap, or other structures, as well as recent bar development. Ratings for this metric are based on the presence of artificial structures as well as the existence, extent, and coarseness of point bars, side bars, and mid-channel bars which indicate the degree of flow fluctuations and substrate stability. Evidence of channelization may sometimes be seen in the form of berms which parallel the stream channel.

g) **Bank Stability** Rated based on the presence/absence of riparian vegetation and other stabilizing bank materials such as boulders and rootwads, and frequency/size of erosional areas. Sites with steep slopes are not penalized if banks are composed solely of stable materials.

h) **Embeddedness** Rated as a percentage based on the fraction of surface area of larger particles that is surrounded by fine sediments on the stream bottom. In low gradient streams with substantial natural deposition, the correlation between embeddedness and fishability or ecological health may be weak or non-existent, but this metric is rated in all streams to provide similar information from all sites statewide.

i) **Channel Flow Status** Rated based on the percentage of the stream channel that has water, with subtractions made for exposed substrates and islands.

j) **Shading** Rated based on estimates of the degree and duration of shading at a site during summer, including any effects of shading caused by landforms.

k) **Riparian Buffer Zone** Based on the size and type of the vegetated riparian buffer zone at the site. Cultivated fields for agriculture which have bare soil to any extent are not considered as riparian buffers. At sites where the buffer width is variable or direct delivery of storm runoff or sediment to the stream is evident or highly likely, the smallest buffer in the segment. (e.g., 0 if parking lot runoff enters directly to the stream) is measured and recorded even though some of the segment may have a well developed buffer. In cases where the riparian zone on one side of the stream slopes <u>away</u> from the stream and there is no direct point of entry for runoff, the buffer on the other side of the stream should be measured and recorded and a comment made in comments section of the data sheet.

l) **Aesthetic Rating** Rated based on the visual appeal of the site and presence/absence of human refuse, with highest scores assigned to stream segments with no human refuse and visually outstanding character.

m) **Remoteness** Rated based on the absence of detectable human activity and difficulty in accessing the segment.

2.3.3 QA/QC for Field Sampling

A Quality Assurance Officer (QAO) experienced in all aspects of the Survey was appointed to administer the quality assurance program. Specific quality assurance activities administered by the QAO included preparing a field manual of standard sampling protocols, designing standard forms for recording field data, conducting field crew training and proficiency examinations, conducting field and laboratory audits, making independent habitat assessments, identifying taxa, reviewing all reports, and reporting errors.

To ensure consistent implementation of sampling procedures and a high level of technical competency, experienced field biologists were assigned to each crew and all field personnel completed program training before participating in field sampling. Training topics included MBSS program orientation, stream segment location using global positioning system (GPS) equipment, sampling protocols, operation and maintenance of sampling equipment, data transcription, quality assurance/quality control, and safety. The spring field crew received additional training in sampling protocols for water quality and benthic macroinvertebrates. The summer field crews received additional training in habitat assessment methods, taxonomy, and *in situ* water chemistry assessment.

Training included classroom, laboratory, and field activities. Instructors emphasized the objectives of the Survey and the importance of strict adherence to the sampling protocols. The QAO conducted proficiency examinations to evaluate the effectiveness of the training program and ensure that the participants had detailed knowledge of the sampling protocols. Members of the spring sampling crew were required to demonstrate proficiency in techniques for collecting samples for water chemistry and benthic macroinvertebrates. At least one member of the summer sampling crew was required to pass a comprehensive fish taxonomy examination. Each crew had to demonstrate proficiency in locating pre-selected stream segments using the GPS receiver and determining if the segment was acceptable for sampling. Comprehensive "dry runs" were conducted to simulate actual field conditions and evaluate classroom instruction.

Field audits were conducted by the QAO during the field sampling to assess the adequacy of training, adherence to sampling protocols, and accuracy of data transcription. The audits included evaluation of the preparation and planning prior to field sampling, stream segment location using GPS equipment and assessment of acceptability for sampling, adherence to sampling protocols, data transcription, and equipment maintenance and calibration. The QAO made an independent assessment of habitat at all segments where field audits were done, approximately 10% of the total number of sites.

2.4 STATISTICAL METHODS

Basins sampled in the MBSS were selected in a probabilistic manner using the lattice design described in section 2.1, so that the stratified random sample of basins could be used for developing both statewide and basin-specific estimates. Within each basin, stream data were collected from a stratified random sample of stream segments as described in section 2.1. The study design allowed for estimation of parameters of interest and biological characteristics, as described below, including mean values and percentage of stream miles exhibiting a characteristic of interest. Because samples were independent and identically distributed within strata, the design also allowed for regression and correlation analyses.

2.4.1 Estimates Based on Stratified Random Sampling (Statewide or Basinwide Estimates)

The observations (y) for segments in the stratified random sample are used to estimate the parameters of interest (e.g., totals, means, proportions, percentiles). The mean for all stream segments in a basin (across stream order) can be estimated as a weighted mean of the sample values. The estimator for the stratified mean of y (e.g., average number of fish per stream segment) is

$$\bar{y}_{st} = \sum W_h \bar{y}_h \quad \textbf{(1)}$$

where $\mathbf{W_h}$ is the number of stream miles of order **h** relative to the total number of stream miles in the basin and $\mathbf{y_h}$ is the mean of y within stream order **h** (Cochran 1977). For example, if there were 348.5 miles of first order streams in the Gunpowder basin out of a total 466.1 first-, second-, and third-order stream miles, $\mathbf{W_1}$ would equal 348.5/466.1 or 0.748.

The estimator for the variance of the stratified mean of y (across stream order) is

$$Var(\bar{y}_{st}) = \sum W_h^2 \frac{s_h^2}{n_h} \quad \textbf{(2)}$$

and

$$s_h^2 = \sum (y_{h,i} - \bar{y}_h)^2 / (n_h - 1) \quad \textbf{(3)}$$

is the sample estimate of the variance in the **h**-th stream order, where $\mathbf{y_{hi}}$ is the value of *y* for segment **i** in stratum **h** (Cochran 1977), and $\mathbf{n_h}$ is the number of samples in the **h**-th stream order.

The above methods were also used to estimate proportions of all stream miles in a basin falling in a given category (e.g., percentage of stream miles in the Upper Potomac basin with ANC < 0 μeq/l) by introducing an indicator variable **I** that takes the value 1 if the observation falls in the specified category, and 0 otherwise. The stratified mean (and standard error) of this indicator variable provides an estimate of the proportion of the population that falls in the category of interest. For stratified random sampling, confidence intervals were derived from the standard errors of the stratified estimates, given that the sample sizes were large enough for the central limit theorem to apply.

2.4.2 Estimates Based on Simple Random Sampling (Within One Stream Order Within a Basin)

Within stream order **h** in a basin, a simple random sample $\mathbf{n_h}$ of segments was selected. Estimates of means (e.g., mean number of fish per segment) are based on the ordinary sample means. If 100% capture efficiency is assumed, the total number or biomass of fish by species is obtained by extrapolating the mean number of fish per segment (combined total from two passes) to the total stream length. In section 2.5, a method is presented for correcting for capture efficiency based on double-pass electrofishing (for details, see Heimbuch et al. 1997).

For simple random sampling, as was used within a stream order within a basin, exact confidence intervals for proportions (or percentages) can be obtained from the binomial distribution. Assume that of the $\mathbf{n_h}$ segments, the number of samples falling in a certain class is $\mathbf{B_h}$ = sum $\mathbf{I_h}$, where the indicator variable $\mathbf{I_h}$ takes the value 1 if the observation falls in the specified category (e.g., ANC < 0), and 0 otherwise. An unbiased estimator of the proportion of segments that falls in the class for the entire stream order in the basin is simply

$$p_h = B_h/n_h,$$

with exact upper and lower confidence limits (Hollander and Wolfe 1973):

$$P_L^{\alpha}(n,\ B) = \frac{B}{B + (n - B + 1)\ f_{\alpha/2,2(n-B+1),2B}} \quad \textbf{(4)}$$

$$P_U^{\alpha}(n,\ B) = 1 - P_L^{\alpha}(n,\ n - B) \quad \textbf{(5)}$$

where **L** and **U** signify the lower and upper confidence limits, **B** is the number of successes in the **n** Bernoulli trials and $\mathbf{f_{\gamma,n1,n2}}$ is the upper γth percentile for **F** distribution with $\mathbf{n_1}$ degrees of freedom in the numerator and $\mathbf{n_2}$ degrees of freedom in the denominator.

2.4.3 Estimation of Biological Characteristics

To estimate biological characteristics for a fish population in a basin (e.g., the size composition of the population of brook trout), the proportions **p** of fish falling into size categories is estimated. Since fish are caught in clusters, statistical methods based on the assumption that samples of individuals are independent, identically distributed, such as binomial or multinomial distributions for estimating proportions, are not valid (Brier 1980, Fay 1985, Roland Thomas and Rao 1987, Skinner et al. 1989). The sampling unit in the electrofishing survey is the individual stream segment, and not the individual fish (Pennington and Vølstad 1994). Therefore, a ratio estimator is used for estimating the proportion of fish within a specific size group (Cochran 1977). The same method is used to estimate the proportion of fish with a specific type of anomaly.

For a species of interest, let $\mathbf{a_{i,h}}$ be the number of fish caught at the **i**-th segment in stream order **h** falling in class **C** (e.g., number of smallmouth bass above legal size), and let $\mathbf{p_{ih}=a_{ih}/y_{ih}}$ where $\mathbf{y_{ih}}$ is the total number of fish caught. A sample estimate of the proportion $\mathbf{p_h}$, falling in class **C** in the population in stratum **h** (Cochran 1977) is

$$p_h = \frac{\sum_{i=1}^{n_h} a_{i,h}}{\sum_{i=1}^{n_h} y_{i,h}} \qquad \textbf{(6)}$$

and an estimate of the variance of $\mathbf{p_h}$ is

$$ar(p_h) = \frac{\sum a_{i,h}^2 - 2p_h \sum a_{i,h} y_{i,h} + p_h^2 \sum y_i}{n_h \overline{y}^2 (n_h - 1)} \qquad \textbf{(7)}$$

where summation is over all segments ($\mathbf{n_h}$) in stratum **h**.

The ratio estimator is biased, but the bias is small for large sample sizes. For small sample sizes (e.g., less than 30), a jackknife estimator would be more efficient (Efron and Gong 1983, Wu and Deng 1983, Pennington and Vølstad 1994). For estimating the proportion falling in class C of the entire population of fish in a basin (i.e., across all stream orders), the stratification of stations needs to be taken into account. The combined ratio estimator (Cochran 1977) was used to estimate proportions of the overall population ($\mathbf{p_{st}}$) in class **C**:

$$p_{st} = \frac{\sum w_h a_h}{\sum w_h y_h} \qquad \textbf{(8)}$$

where for the **h-**th stratum $\mathbf{w_h}$ is the proportion of the stream length in the stratum, $\mathbf{a_h}$ is the total number of fish in class **C** caught in the stratum, and $\mathbf{y_h}$ is the total number of fish (all classes) caught in the stratum. The variance of $\mathbf{p_{st}}$ is estimated by jackknifing (Saerndal et al. 1992).

2.5 CAPTURE EFFICIENCY ADJUSTMENT FOR FISH POPULATION ESTIMATES

Estimates of fish density (number of individuals per stream mile) and total abundance (number of individuals per basin) were corrected for capture efficiency using an analytical technique developed with the 1995 MBSS data. This method used electrofishing catch data to estimate actual density and population size based on the rate of decline in catch per unit effort over the two passes. Typically, it is difficult to make estimates of capture efficiency with a small number of passes from a single site because of the likelihood, for some fish species, of collecting on the second pass an equal or greater number of fish than on the first pass. To address this problem, this new method pooled samples over multiple stream segments within the same stream order and basin. Using a modified Seber-LeCren estimator (Seber 1982, Seber and LeCren 1967), this technique analytically corrected for bias introduced by variable probability of capture and minimized bias typically resulting from small sample size. The capture efficiency adjustment method is described fully in Heimbuch et al. (1997) and Roth et al. (1997).

2.6 LANDSCAPE ANALYSIS

Land uses within watersheds upstream of sample sites were derived with a geographic information system (GIS), using Micro Images (MIPS) and PC Arc Info software. Watersheds upstream of each sample site were digitized using topographic lines from digital county topographic maps (1:62,500 scale). Watersheds were digitized in TNT MIPS and exported to PC Arc Info. The watershed file was then intersected with land use/land cover information from the Federal Region III Multi-Resolution Land Characteristics (MRLC) digital data set, Version 2 (MRLC 1996a,b). The MRLC was developed by a federal agency consortium, using data primarily from Landsat 1991-93 Thematic Mapper satellite images at a resolution of 30 x 30 m pixels. The MRLC classifies land cover into 15 categories (Table 2-4). Using GIS, the area within each watershed was calculated as was the percentage of area within each watershed represented by each type of land use. For some analyses, land uses were collapsed to the following six classes: water, urban land, agriculture, forest, wetlands, and barren.

Table 2-4. Land cover classes in the Multi-Resolution Land Characteristics data set for Region III (Version 2, MRLC 1996a, b). Percentages given in class definitions should be viewed as guidelines.

Water

Open Water **-** all areas of open water, generally with less than 25 percent vegetation or other land cover.

Developed Land

Low Intensity Developed **-** Land includes areas with a mixture of constructed materials and vegetation or other cover. Constructed materials account for 30-80 percent of the total area. Commonly includes single-family housing areas, such as suburban neighborhoods.

High Intensity Developed **-** Includes heavily built-up urban centers and large constructed surfaces in suburban and rural areas. Vegetation occupies less than 20 percent of the landscape. Constructed materials account for 80-100 percent of the total area. Examples include apartment complexes, skyscrapers, shopping centers, factories, industrial complexes, airport runways, and interstate highways.

Herbaceous Planted / Cultivated

Hay / Pasture / Grass **-** Grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops. Also includes golf courses and city parks.

Row Crops **-** All areas used for the production of crops, such as corn, soybeans, vegetables, tobacco, and cotton.

Probable Row Crops **-** Areas of row crop that may be confused with other areas, such as grasslands that were not green during times of spring data acquisition.

Natural Forested Upland

Deciduous Forest **-** Areas dominated by trees where 75 percent or more of the tree species shed foliage seasonally.

Evergreen Forest **-** Areas dominated by trees where 75 percent or more of the tree species maintain their leaves all year. Canopy is never without green foliage.

Mixed Forest **-** Areas dominated by trees where neither deciduous nor evergreen species represent more than 75 percent of the cover present.

Wetlands

Woody Wetlands **-** Areas of forested or shrubland vegetation where the soil or substrate is periodically saturated with or covered with water as defined by Cowardin et al. (1979).

Emergent Herbaceous Wetlands **-** Non-woody vascular perennial vegetation where the soil or substrate is periodically saturated with or covered with water as defined by Cowardin et al.(1979)

Barren

Quarries / Strip Mines / Gravel Pits **-** Areas of extractive mining activities with significant surface expression.

Coal Mines **-** Areas dominated by spectrally dark coal piles and strip mines.

Bare Rock/Sand/Clay **-** Includes areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, beach, and other accumulations of rock and/or sand without vegetative cover.

Transitional **-** Areas dynamically changing from one land cover to another, often because of land use activities. Examples include forest lands cleared for timber, and may include areas freshly cleared or in early stages of forest regrowth.